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SCHOOL OF AVIATION MEDICINE  
RANDOLPH AIR FORCE BASE, TEXAS

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**AORTAL CHOLESTEROL CONCENTRATIONS AFTER LINODOXINE ADMINISTRATION IN DOGS**

**LAWRENCE J. MILCH, Colonel, USAF  
LESLEY G. ROBINSON, Staff Sergeant, USAF  
SHERMAN S. WILSON, M.S.  
FREDRICK H. WILSON, JR., B.S.**

Department of Pharmacology and Biochemistry



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**Air University  
SCHOOL OF AVIATION MEDICINE, USAF  
RANDOLPH AFB, TEXAS**

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## AORTAL CHOLESTEROL CONCENTRATIONS AFTER LINODOXINE ADMINISTRATION IN DOGS

During the past three years a number of drug preparations containing linoleic acid have been marketed by the pharmaceutical industry. Ethical advertising of these substances has referred (1) to extensive data demonstrating the hypocholesterolemic effect of essential unsaturated fatty acids in man, and (2) to the statistical inference that lowered blood cholesterol levels will contribute to reduced arterial "atherogenesis." Hegsted et al. (3) reported that corn oil (55.7 percent linoleic acid) administration resulted in lowered serum cholesterol concentrations and less vascular sudanophilia in the cholesterol-cholic acid-fed rat, but failed to demonstrate any effect of the unsaturated essential fatty acid administration on cholesterol deposition at the arterial wall. Since arterial cholesterol deposition may be more closely related to the pathogenesis of atherosclerosis than are serum cholesterol levels, it was decided to determine any effect of Linodoxine<sup>1</sup> on the cholesterol concentrations of the aortae and coronary arteries of dogs fed a hypercholesterolemic regimen of excess fat, cholesterol, and propylthiouracil.

### EXPERIMENTAL

Two groups (L and P) of 6 dogs each were fed identical diets of Ken-L-Ration, lard, cholesterol, and propylthiouracil over a 9- to 11-week period. The diet of the group L dogs was supplemented by the daily addition of 200 cc. of Linodoxine while the group P animals received an equal volume of a placebo mixture prepared by the manufacturer to be similar in taste, appearance, and consistency to Linodoxine. The placebo mixture differed significantly from the Linodoxine in that (1) coco-

nut oil<sup>2</sup> was substituted for safflower oil, and (2) neither pyridoxine nor mixed tocopherols were incorporated. A third group (C) of 6 dogs was maintained over the experimental period on Ken-L-Ration, lard, and propylthiouracil. All animals were presented with equal quantities of the appropriate ration twice daily. No effort was made to control food intake, and water was allowed ad libitum. The food mixtures were well tolerated and individual weightings of the animals at weekly intervals failed to demonstrate significant change. The diet mixtures, including supplements, were repeatedly analyzed for cholesterol and fat during the experimental period. Cholesterol assays yielded values as follows: diets L and P, 220 mg. percent; diet C, 80 mg. percent. Fat<sup>3</sup> assays: L, 17.5 percent; P, 20.5 percent; C, 10.8 percent. The absence of gross signs of myxedema in the animals suggested that the propylthiouracil had a minimal antithyroid effect.

Control blood samples were secured from each dog prior to beginning the dietary regimens, and the sampling was repeated at 14-day intervals. Serum assays for cholesterol, lipid phosphorus, and the ultracentrifugal lipoproteins were accomplished on each blood sample (4). When the average peak concentrations (6th week) of total serum cholesterol were passed, as indicated by measurements at the 8th week, serial sacrifice of the dogs was initiated. One animal from each group was sacrificed by rapid arterial exsanguination on Monday, Wednesday, and Friday of the 10th and 11th weeks. Aortae and coronary arteries were excised, freed of adhering fat and connective

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<sup>1</sup>Safflower oil — 78 percent linoleic acid, 10 percent oleic acid, and 12 percent saturated fatty acids.

<sup>2</sup>Two percent linoleic acid, 7.3 percent oleic acid, and 90.7 percent saturated fatty acids.

<sup>3</sup>Gravimetric determinations for fat, involving extraction with petroleum ether and evaporation to dryness, may be expected to give falsely low values for the more labile unsaturated oils, thus accounting for the analytic difference between diets L and P.

tissue, and analyzed for cholesterol concentration (5). A portion of each artery was reserved for histologic examination.

## RESULTS AND DISCUSSION

Histologic examination of aortae and coronary arteries revealed several instances of intimal hyperplasia and occasional fragmentation of the internal elastic membrane consistent with atherosclerosis, but the pathosis was randomly distributed throughout the animal groups. Apparently such minimal changes as were observed existed in these dogs prior to the experiment and were thus unrelated to the dietary regimens. Chemical analyses of the coronary arteries yielded identical averages for cholesterol concentrations — 1.56 mg./gm. wet weight for each of the three groups of dogs. This figure was also obtained as the average for 26 control dogs sacrificed at two-week intervals over a year's period and reported previously (5). Such suggests that the varying dietary regimens failed to influence the cholesterol concentrations of the coronary arteries.

A somewhat different result was obtained when the dog aortae were subjected to chemical analysis for cholesterol. On each sacrifice date (fig. 1) the cholesterol concentration of the aorta taken from the dog after Linodioxine administration (group L) was lower ( $P < .05$ ) than the aortal concentration observed in the corresponding animal maintained on the placebo supplement. Excepting the single value obtained for the control (group C) dog on 19 November, aortal cholesterol concentrations recorded for all animals are consistent with the hypothesis that a Linodioxine supplement to a diet containing excess fat and cholesterol will inhibit any associated increased aortal deposition of cholesterol.

This experiment fails to identify the component that confers such suggestive activity on the Linodioxine mixture. It must be noted, however, that although pyridoxine and mixed tocopherols were absent from the prepared placebo, these vitamins are present in abun-

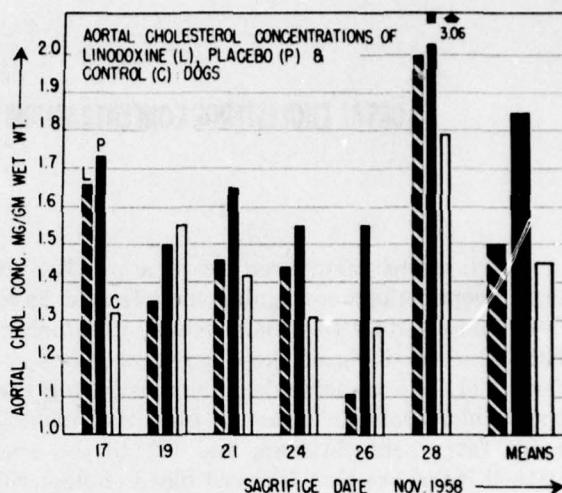


FIGURE 1

Aortal cholesterol concentrations of Linodioxine (L), placebo (P) and control (C) dogs.

dance in Ken-L-Ration. The remaining difference between the Linodioxine and the placebo is the character of the fatty acids contained in each. Any definitive conclusion on such sparse data becomes hazardous, indeed, but these findings suggest that a suitable mixture of dietary fatty acids may, in fact, be antiatherogenic.

Serum total cholesterol and lipid phosphorus concentrations, obtained as a result of serial blood sampling of each dog, were virtually indistinguishable between groups fed Linodioxine and placebo supplements. Neither the cholesterol/lipid phosphorus ratio nor that fraction of total cholesterol transported as  $\beta$ -lipoprotein<sup>4</sup> was consistently altered in the Linodioxine-fed dogs. Further fractionation of the  $\beta$ -lipoproteins in the analytic ultracentrifuge, however, was somewhat more fruitful. Pronounced trends

toward increased levels of the  $S_f^{0-12}$  classes and simultaneous decreases of the  $S_f^{12-400}$  classes of serum lipoproteins were associated

<sup>4</sup>All lipoproteins with densities  $\geq 1.063$  are isolated in preparative ultracentrifuge and analyzed for cholesterol.



TABLE I

Ratios of lipoprotein concentrations  $\frac{S_f^0 \text{ 12-400}}{S_f^0 \text{ 0-12}}$ ; prediet values (PV), average maintenance values during the dietary period (MV), and net changes ( $\Delta$ ) for Linodoxine, placebo, and control dogs

Linodoxine supplement			Placebo supplement			Controls		
PV	MV	$\Delta$	PV	MV	$\Delta$	PV	MV	$\Delta$
.304	.221	-.083	.082	.649	.567	.393	.241	-.152
.026	.348	.322	.214	.222	.008	.000	.402	.402
.338	.087	-.251	.099	.355	.256	.039	.319	.280
.383	.107	-.276	.041	.512	.471	.052	.070	.018
.153	.288	.135	.138	.320	.182	.280	.356	.076
.104	.311	.207	.047	.563	.516	.094	.126	.032
Av. .218	.227	.009*	.103	.438	.333†	.143	.252	.109*

\*Not significant.

†Highly significant ( $P < .01$ ).

with Linodoxine administration. When these

values were combined into a ratio  $\frac{S_f^0 \text{ 12-400}}{S_f^0 \text{ 0-12}}$ ,

significant discrimination between Linodoxine dogs and placebo dogs could be recorded. These data (table I) indicate a uniform plus change

in the ratio  $\frac{S_f^0 \text{ 12-400}}{S_f^0 \text{ 0-12}}$  between the control

sample and the mean of all observations taken during the period of experimental feeding among the dogs in the placebo group. Linodoxine was apparently associated with a reversal of this trend, inasmuch as control-dietary changes in the ratio for the Linodoxine-fed dogs are statistically indistinguishable from those recorded for the group C animals.

## SUMMARY

Linodoxine was added to the diet of 6 dogs fed excess fat, cholesterol, and propylthiouracil over a 9- to 11-week period. Administration of this drug mixture was associated with significantly ( $P < .05$ ) decreased concentrations of aortal cholesterol as compared to a simultaneously maintained group of dogs fed the same diet containing a placebo mixture of coconut oil in place of Linodoxine. Linodoxine was also associated with a significant shift of serum  $\beta$ -lipoproteins toward classes of higher density, a reversal of the trend noted in the placebo-fed group of dogs.

Pathologic examination of arterial tissue described in this study was accomplished by Captain Robert A. Brooks, Department of Pathology.

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